OSMOREGULATORY ROLE OF THE ANTENNARY GLAND IN TWO SPECIES OF ESTUARINE CRABS [1]

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Osmotic regulation in aquatic animals has been reviewed by Krogh (1939), Beadle (1957) and discussed more recently by Prosser and Brown (1961) and Lockwood (1962). Beadle (1957, p. 335) has commented on the evolution of osmotic regulation in Crustacea, and postulated "that in marine crabs there are at least two sets of active processes at work, in the gills and in the excretory organs, which are responsible for the ionic imbalance between blood and sea water." He suggested that adjustments in the rates of these processes could have led, in appropriate environments, to the evolution of both hypo- and hyper-osmotic regulation. Osmotic behavior of nine species of eastern Pacific crabs was investigated by Jones (1941), who categorized *Hemigrapsus oregonensis* and *H. nudus* as hyperosmotic regulators, without any capacity for hypo-osmotic regulation. Gross (1961) recently reported *Hemigrapsus oregonensis* to be a strong hypo-osmotic regulator in sea water as high as 175%. The mechanisms by which crabs establish and maintain osmotic and ionic gradients between their internal and external environments have been studied (Nagel, 1934; Green, Harsch, Barr and Prosser, 1959). In *Carcinus maenas*, a crab showing no hypo-osmotic regulation, Prosser and Brown (1961, p. 14) have suggested three mechanisms which play a part in hyper-osmotic regulation: "low permeability to water and salts, increased fluid output, particularly of urine, and active salt absorption from the medium." Participation of the antennary glands in hyper-osmotic regulation in *Pachygrapsus crassipes*, a species which regulates in both low and high salinities, has been suggested (Prosser, Green and Chow, 1955). Gross (1957a) has submitted that active absorption of water may be a method of hypo-osmotic regulation. The antennary glands have been considered to be more important in ionic than total osmotic regulation (Prosser, Green and Chow, 1955; Green, Harsch, Barr and Prosser, 1959; Prosser and Brown, 1961). Evidence for this viewpoint has been obtained mainly from the work of Nagel (1934), Webb (1940), Robertson (1949) and Parry (1954). In a semi-terrestrial crab, *Coenobita perlatus*, Gross and Holland (1960) demonstrated behavioral mechanisms for regulation of osmotic concentration of the blood. The antennary glands in this species were shown to contribute only to the regulation of potassium and not total osmotic regulation. The ratio, urine concentration/blood concentration (U/B ratio), for specific ions in selected regulating and adjusting

Crustacea, has indicated that the antennary glands do act selectively to control certain ionic imbalances between blood and external media (Peters, 1935; Picken, 1936; Prosser, Green and Chow, 1955; Gross, 1959; Gross and Holland, 1960; Prosser and Brown, 1961).

The effects of temperature on osmotic regulation in various aquatic organisms have been investigated (Wikgren, 1953; Dehnel, 1962) and reviewed, in part, by Verwey (1957). The effect of external salinity on animal activity, particularly on osmotic behavior and water and ion fluxes between body fluids and media, and concomitant weight changes have been studied (Jones, 1941; Robertson, 1949, 1953; Gross, 1954, 1955, 1957a; Prosser, Green and Chow, 1955; Dehnel, 1960, 1962).

The osmotic behavior of *H. oregonensis* and *H. nudus* has been studied with respect to blood responses over a range of experimental temperatures and salinities (Dehnel, 1962). These two species are established in this geographical area with seasonal temperature and salinity cycles similar to those discussed by Broekema (1941) and Verwey (1957). The activity of the antennary glands in *Hemigrapsus* demonstrates that the osmoregulatory responses of these species change significantly with seasonal field temperature and salinity.

Material and Methods

Two species of shore crabs, *Hemigrapsus nudus* (Dana) and *H. oregonensis* (Dana), were collected at two seasons, summer and winter, from the intertidal zone at Spanish Bank, Vancouver, British Columbia.

In the laboratory the animals were divided into four groups, and depending on size, experimental salinity and temperature, the number per plastic tray varied from 10 to 15 animals. Each group provided three sequential sets of 10 separate urine samples.

To bring the animals to a common osmotic level each group was totally immersed for 36 to 48 hours in 75% sea water, a suitable intermediate salinity for equilibration. Trays were placed in darkened refrigerators set at the experimental temperature. Following equilibration, each group was transferred to 4.0 liters of water at experimental temperature and salinity conditions. Animals were not fed and sea water was renewed daily.

Experimental temperatures, summer and winter, were 5°, 15°, and 25° C. (±1.0° C.), and experimental salinities were 6%, 12%, 25%, 75%, 100%, 125%, 150% and 175% sea water, based on a standard sea water: 31.88‰ salinity and 17.65‰ chlorinity at 25° C. Experimental salinities were obtained either by diluting sea water with distilled water or by concentrating it with appropriate amounts of sea salt. Salinities were determined by a 1000-cycle conductivity bridge calibrated to the standard sea water, and alternatively by titration. Field and experimental salinities are expressed as percentage sea water based on the above standard.

After 3, 24 and 48 hours at the experimental conditions, urine was sampled by means of glass capillary tubes, 0.40 mm. inside diameter and 1½ inches long, drawn to a fine tip and inserted into a small rubber pipette bulb. Crabs were blotted dry and manipulated under a binocular microscope, so that the tip of a

blunt needle, mounted on the microscope stage, could be inserted under the operculum, which covers the pore. As the operculum was raised, the tip of the capillary tube was inserted beneath it. This usually resulted in a discharge of urine. If necessary, gentle pressure, exerted dorso-ventrally on the body of the crab, would cause expulsion of urine. The tubes were sealed immediately with "Seal-Ease" and quick-frozen on dry ice. Samples were then transferred to a brine solution at $-15°$ C., until needed. The animals were returned to the ex-

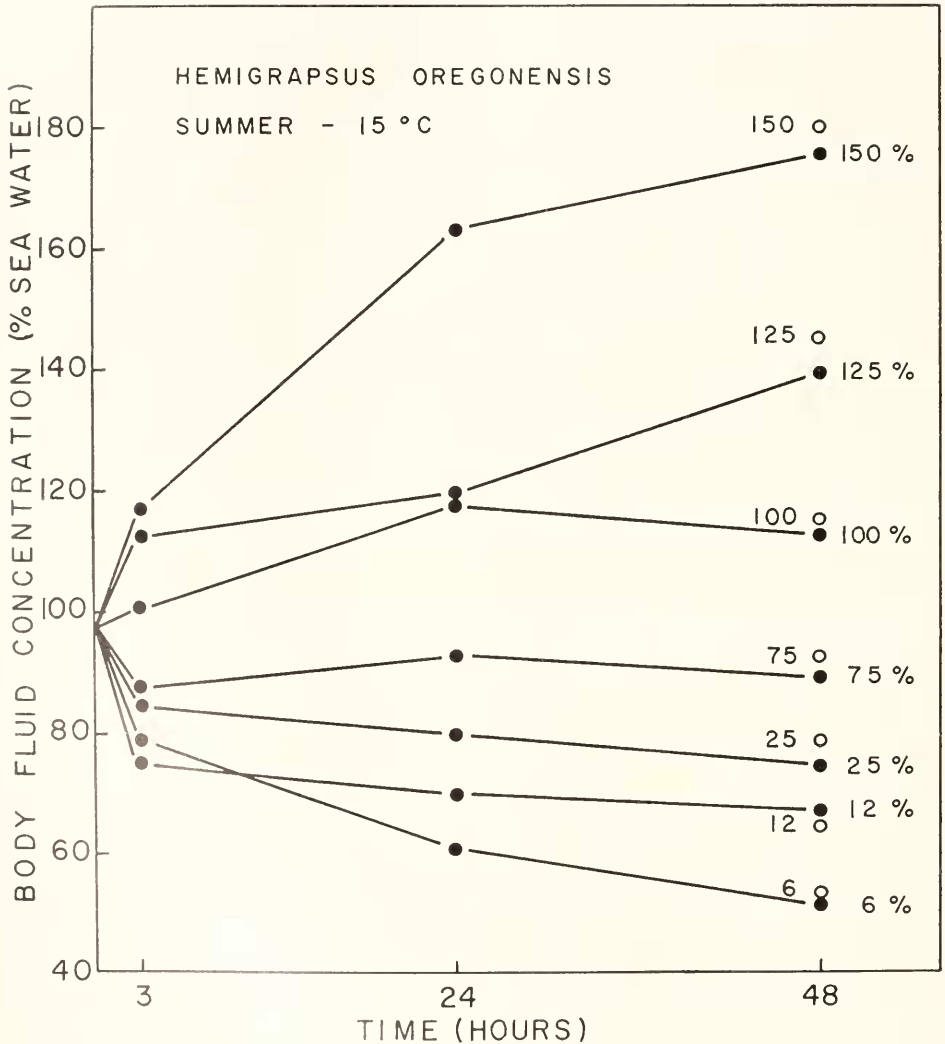FIGURE 1. Urine concentration changes and 48 hour blood values in summer *Hemigrapsus oregonensis*, at 15° C. as a function of time in the experimental salinities. Each point represents the mean of the measurements of 10 animals. Urine values are indicated by closed circles (●), blood values (Dehnel, 1962), by open circles (○).
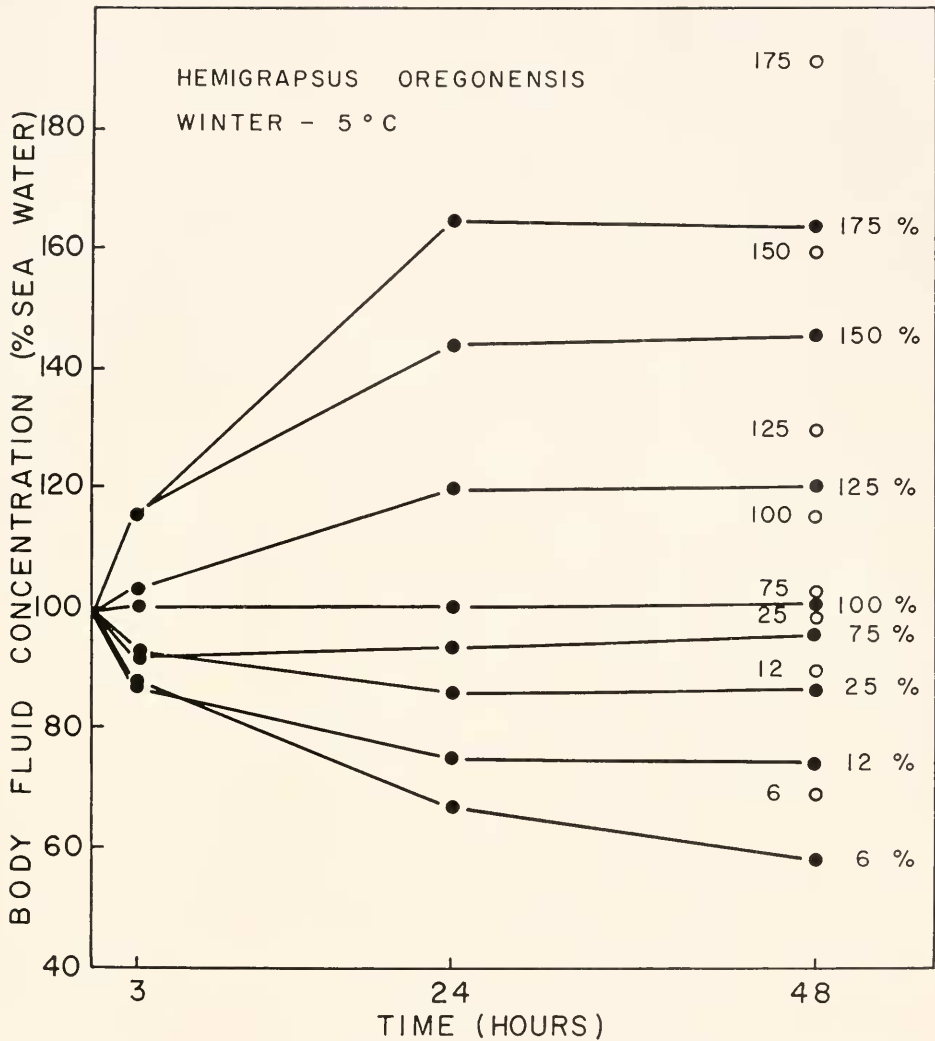
FIGURE 2. Urine concentration changes and 48-hour blood values in winter *Hemigrapsus oregonensis*, at 5° C., as a function of time in the experimental salinities. Each point represents the mean of the measurements of 10 animals for each time period. Urine values are indicated by closed circles (●), blood values (Dehnel, 1962), by open circles (○).

perimental conditions after the 3-hour and 24-hour samplings, and discarded after 48 hours. Measurement of the total osmotic concentration of urine samples was accomplished by the method of melting point determination described by Jones (1941) and modified by Gross (1954).

The data were analyzed for salinity and temperature effects and seasonal differences by means of Student's "t" test. The same test was applied to the differences between mean osmotic concentrations of blood (Dehnel, 1962) and urine

from similarly treated animals. Unless otherwise stated, statistical significance is
attributed to P values <0.01.

RESULTS

Response to external salinity changes

Osmotic responses of *H. oregonensis* and *H. nudus* to a range of experimental
salinities are shown in Figures 1 to 4. Forty-eight-hour blood concentration values
(Dehnel, 1962) are included for comparison. In each figure, urine concentration
at time zero is an arbitrary mean of all three-hour values used in the figure. The
3-, 24- and 48-hour values for animals in 75% sea water were obtained after 36
to 48 hours' equilibration in that medium. Responses common to the four sets of
curves are the rise in urine concentration with time in high salinities, and the fall
in low salinities, at rates directly related to the gradients between media and urine
concentrations at time zero.

Hemigrapsus oregonensis

Summer animals (Fig. 1) did not survive 48 hours in 175% sea water. In
general, changes in blood and urine concentrations were rapid, and a steady-state

TABLE I

*Comparison of 48-hour urine and blood concentrations (urine/blood ratio and urine minus blood
gradient) for summer-adapted and winter-adapted animals at three experimental
temperatures, 5°, 15° and 25° C., and three experimental salinities, 12%,
75% and 125% sea water. P values apply to differences between
concentration of blood and urine*

		Summer								
Exp. sal. (% S.W.)	(°C.)	12%			75%			125%		
		U/B	U-B	P value	U/B	U-B	P value	U/B	U-B	P value
H. nudus	5	0.84	−11.7	<0.010	0.97	−2.4	N.S.	0.94	−8.3	N.S.
H. oreg.	5	0.90	−6.2	N.S.	0.99	−1.3	N.S.	—	—	—
H. nudus	15	1.01	0.9	N.S.	0.99	−0.6	N.S.	0.98	3.3	N.S.
H. oreg.	15	1.02	1.3	N.S.	0.95	−4.6	<0.001	0.96	−5.7	<0.01
H. nudus	25	0.98	−1.0	N.S.	0.98	−1.7	N.S.	0.98	−2.4	N.S.
H. oreg.	25	0.98	−1.1	N.S.	1.04	3.3	N.S.	1.00	−0.2	N.S.
		Winter								
H. nudus	5	0.75	−19.3	<0.001	0.83	−19.8	<0.001	0.83	−24.3	<0.001
H. oreg.	5	0.83	−15.1	<0.001	0.95	−5.0	N.S.	0.93	−9.4	<0.005
H. nudus	15	0.67	−21.9	<0.001	0.97	−2.5	N.S.	0.77	−33.6	<0.001
H. oreg.	15	0.63	−31.1	<0.001	0.77	−22.9	<0.001	0.86	−17.7	<0.001
H. nudus	25	0.63	−28.3*		0.87	−12.7*		0.82	−24.4	<0.001
H. oreg.	25	0.69	−25.1*		0.87	−12.1	<0.001	0.88	−15.4	<0.001

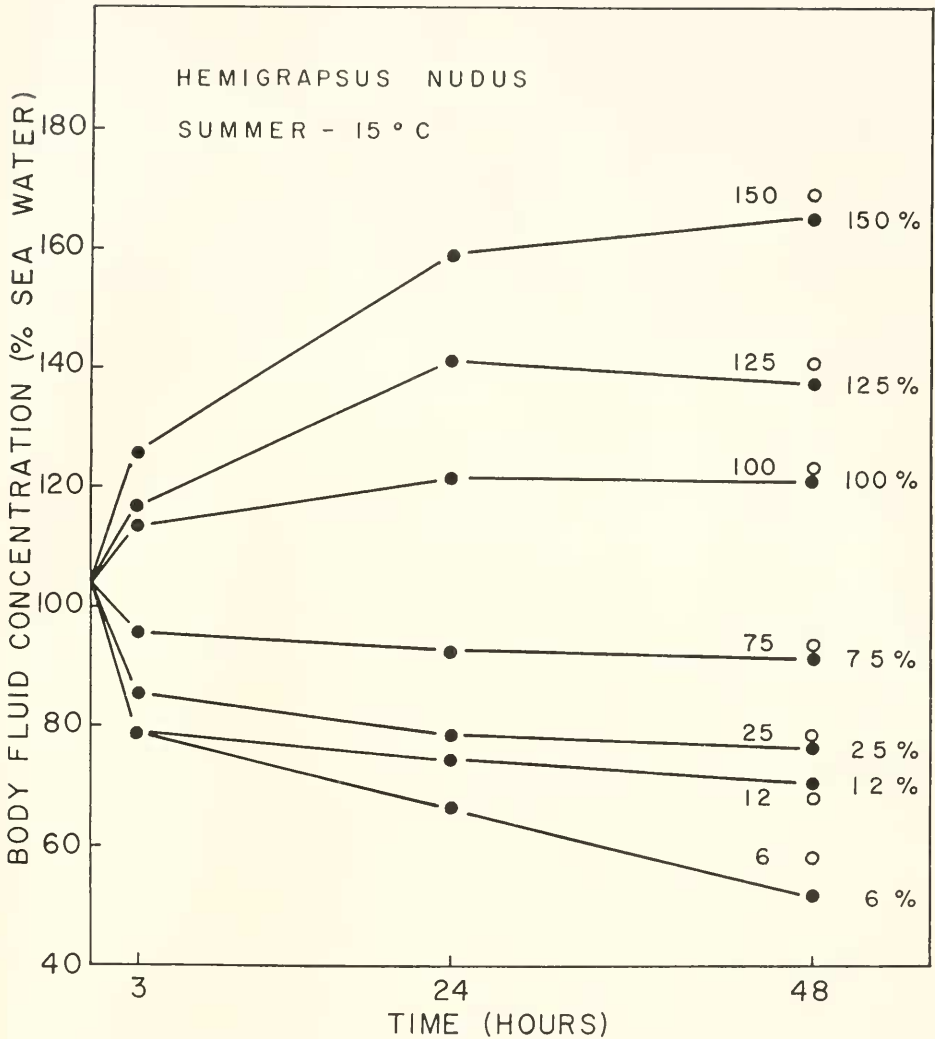* = 24-hour blood values.
N.S. = not significant.

FIGURE 3. Urine concentration changes and 48-hour blood values in summer *Hemigrapsus nudus,* at 15° C., as a function of time in the experimental salinities. Each point represents the mean of the measurements of 10 animals for each time period. Urine values are indicated by closed circles (●), blood values (Dehnel, 1962), by open circles (O).

was reached after 24 hours' exposure, with few exceptions. It was assumed, however, that all major changes in the concentrations of both body fluids had occurred at the end of 48 hours. Urine concentrations were hyper-osmotic to high salinities. Blood and urine was isosmotic, except at 75% and 125% sea water, where the blood was significantly higher (Table I). Hyper-osmoticity of the urine in high salinities was probably the result of continued absorption of salts by gill and gut
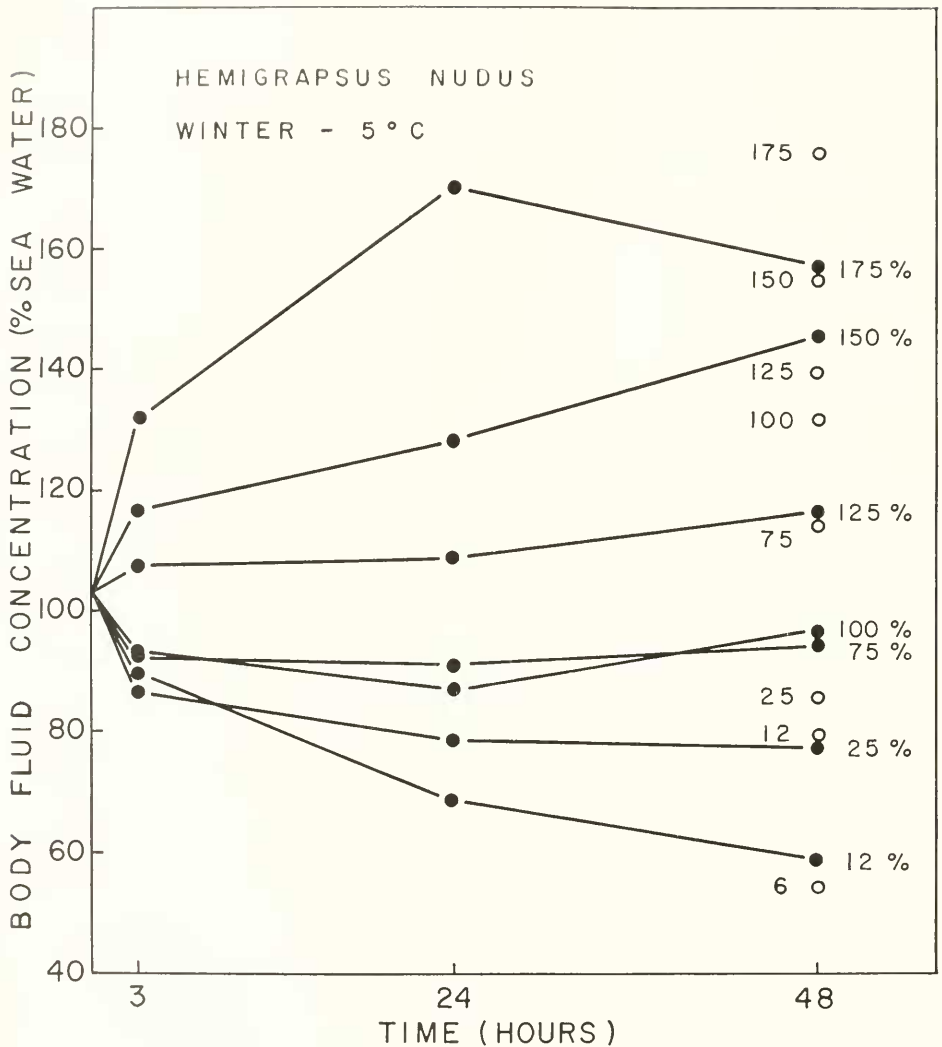
FIGURE 4. Urine concentration changes and 48-hour blood values in winter *Hemigrapsus nudus*, at 5° C., as a function of time in the experimental salinities. Each point represents the mean of the measurements of 10 animals for each time period. Urine values are indicated by closed circles (●), blood values (Dehnel, 1962), by open circles (○).

tissues and a concomitant loss of water to the external media through the integument. In salinities below 100% sea water hyper-osmotic regulation occurred.

Winter animals (Fig. 2) survived 48 hours in 175% sea water, probably reflecting adaptation to higher salinity field conditions. Again, the blood and urine curves reached equilibration after 24 hours. In high salinities, urine was hypoosmotic to both the blood and the media. As in summer animals, at salinities below 100%, hyper-osmotic regulation occurred.

Hemigrapsus nudus

Urine and blood concentration changes occurred in this species at the same rate as in *H. oregonensis*. Summer animals (Fig. 3) survived in 150% but not in 175% sea water. Blood and urine were isosmotic (Table I), and both fluids were hyper-osmotic to all experimental salinities. In winter animals (Fig. 4) the separation of the urine curves, especially in 75% and 100% sea water, was not as clear as in the summer experiments. Animals survived 48 hours in 175%. The lower sur-vival limit of animals from which urine was collected was 12%, and the 48-hour concentration was significantly lower than the comparable summer value. Urine concentrations were hypo-osmotic to the media in high salinities, whereas blood concentrations were hyper-osmotic, but approached isosmoticity at 150% and 175% sea water. Over the entire salinity range blood concentrations were significantly higher than urine.

Interspecific comparison

The absolute difference between 48-hour urine concentrations for *H. oregonen-sis* in 6% and 150% sea water was 125% in summer and 88% in winter. For *H. nudus*, the difference in 12% and 150% sea water was 92% in summer and 86% in winter. In both species higher summer urine concentrations in media above 100% sea water accounted for this difference. A similar comparison for blood resulted in an identical difference for *H. oregonensis*, summer and winter, and for *H. nudus* the differences were approximately the same (100% in summer, 76% in winter).

In summer, blood and urine concentrations for *H. nudus* showed a more con-stant response than *H. oregonensis* in the high salinities. This suggests that *H. nudus* potentially is the better regulator in high salinities. In low salinities, the abilities of both species to hyper-osmoregulate were similar. In winter, *H. ore-gonensis* showed the more constant response to high salinities, and regulated to a greater degree in salinities lower than 100%.

Seasonal effect of salinity

The abilities of the two species to establish and maintain osmotic gradients be-tween their body fluids and the external media changed from summer to winter. To evaluate seasonal effects, gradients were derived from the data of Figures 1 to 4 and from Dehnel (1962).

Hemigrapsus oregonensis

When the gradient between blood concentration and experimental sea water is compared, winter crabs maintained a significantly greater gradient below 100% sea water; above 100%, summer crabs maintained the greater gradient (Fig. 5). Winter urine was hyper-osmotic to summer urine in low salinities, and above 100% sea water, hypo-osmotic to summer urine and the media.

Comparison of the seasonal gradients between blood and urine concentrations is given in Figure 6. At all salinities except 75% the winter gradient is signifi-

cantly higher. This is mainly accounted for by the fact that winter blood maintains a proportionately greater gradient relative to the experimental salinities, and to the urine, whereas summer blood and urine are essentially isosmotic. Winter crabs would appear to be better regulators in hypo-osmotic media, and summer crabs, in hyper-osmotic media (Fig. 5). Further, the isosmotic condition be-
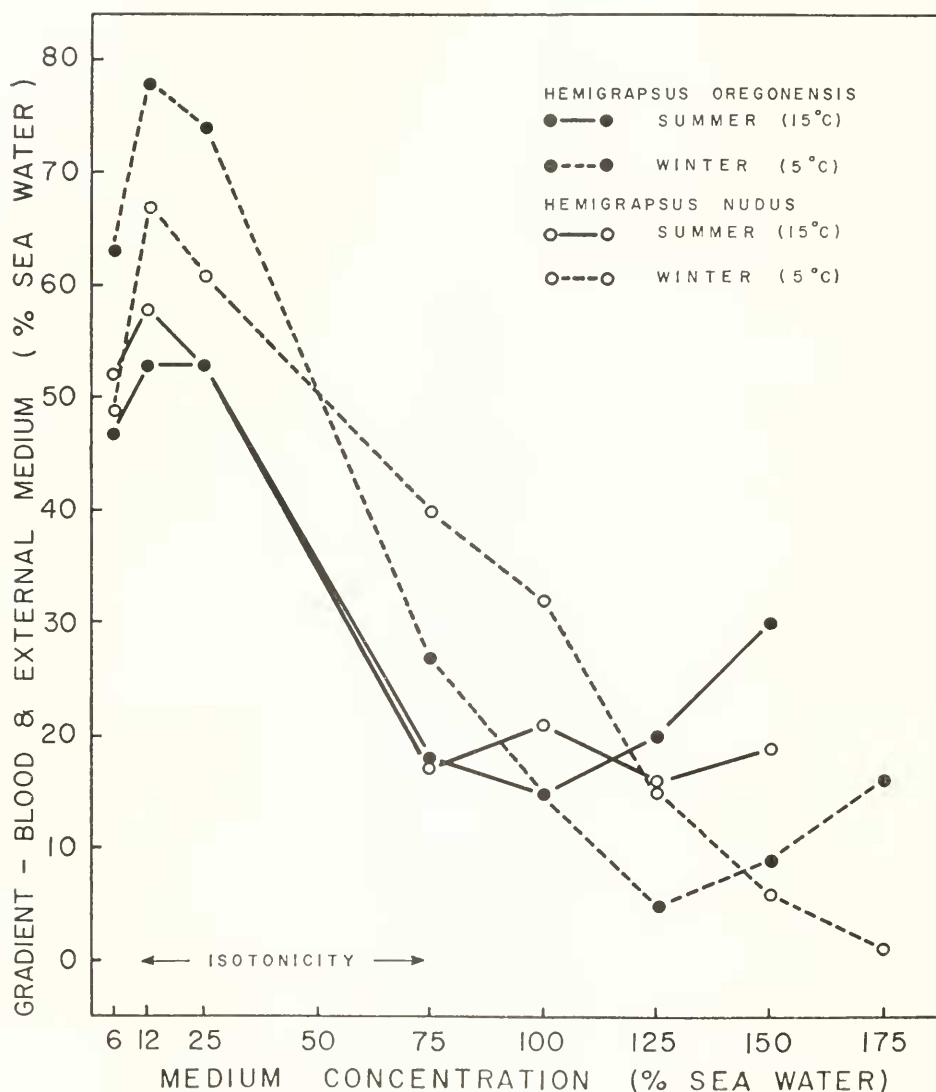

FIGURE 5. Relationship of the gradient between blood and external sea water to medium concentrations in summer (15° C.) and winter (5° C.) *Hemigrapsus oregonensis* and *Hemigrapsus nudus,* after exposure for 48 hours to the experimental salinities. For the purposes of comparison, blood data have been inserted from a previous publication in this journal (Dehnel, 1962).
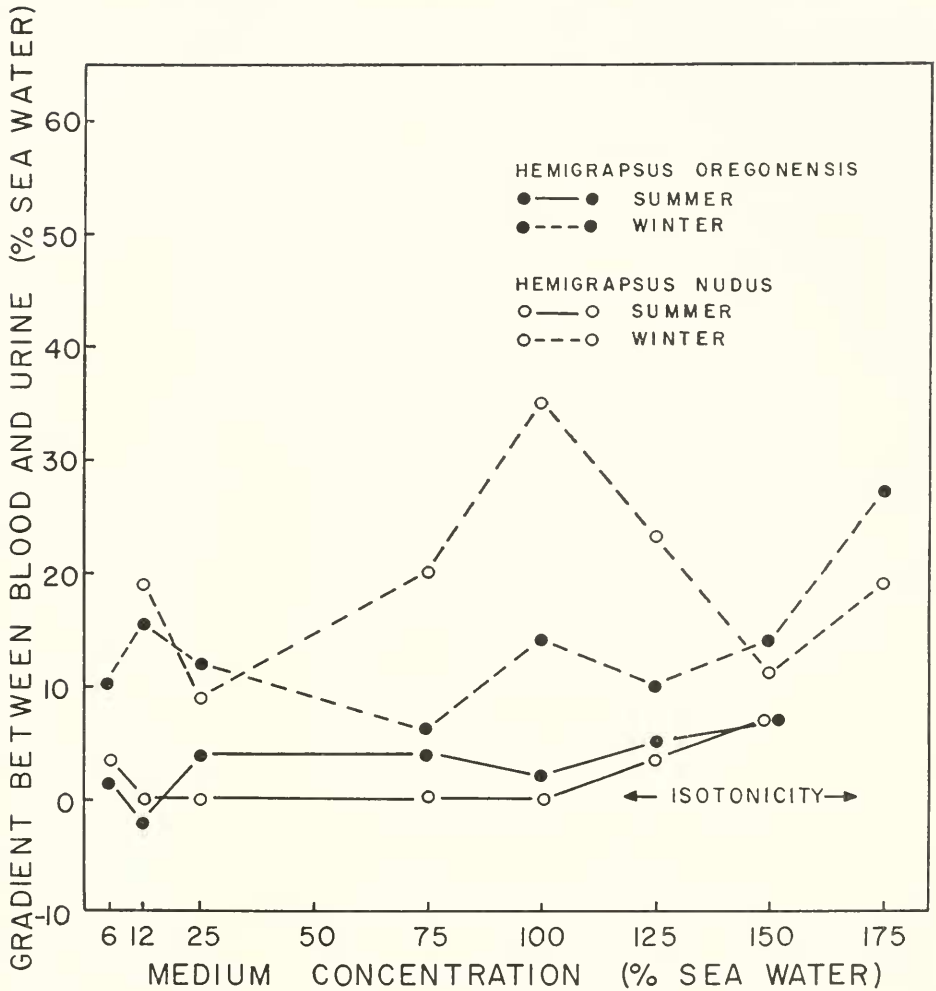
FIGURE 6. Relationship of the gradient between blood and urine to medium concentrations in summer (15° C.) and winter (5° C.) *Hemigrapsus oregonensis* and *Hemigrapsus nudus*, after exposure for 48 hours to the experimental salinities. Blood values are from Dehnel (1962).

tween summer blood and urine implicates extra-renal mechanisms in hyper-osmotic regulation. Production of hypo-osmotic urine by winter-adapted animals suggests the participation of the antennary glands in hyper-osmotic regulation.

Hemigrapsus nudus

The results for *H. nudus* in general paralleled those for *H. oregonensis*. The blood concentration gradient for winter crabs was the greater below 125% sea water, and above this, summer crabs maintained the greater gradient (Fig. 5). As

salinity increased, winter crabs steadily decreased their gradient, and attained isosmoticity at 175% sea water. Summer and winter urine from 25% to 75% sea water was isosmotic. At higher salinities summer urine was significantly hyperosmotic to winter urine and to the media. Winter urine was isosmotic at 100% and significantly hypo-osmotic at higher salinities.

Blood and urine comparison (Fig. 6) shows that at all salinities the winter gradient was significantly higher. Again, the higher winter gradient can be accounted for by the fact that summer blood and urine are isosmotic. From these data it would appear that winter crabs are the better regulators.

Interspecific comparison

Winter blood concentration gradients of both species were higher than corresponding summer ones at salinities below approximately 100% sea water. Above that salinity summer crabs had higher gradients and winter crabs approached isosmoticity (Fig. 5). Urine concentration gradients in general were similar to those of the blood, except that absolute differences were less in low salinities and greater in high salinities. Blood-urine differences for winter crabs were, in general, much greater for *H. nudus*, whereas summer differences were much less, and *H. oregonensis* tended to maintain the greater difference between blood and urine (Fig. 6).

TABLE II

*Seasonal comparison for both species of 48-hour urine (upper values) and blood (lower values)
concentrations of summer-adapted animals (S) and winter-adapted animals (W) at 5°,
15° and 25° C., measured at a series of experimental salinities*

Experimental salinities	(°C.)	Body fluid concentration (% S.W.)											
		12%		25%		75%		100%		125%		150%	
		S	W	S	W	S	W	S	W	S	W	S	W
H. nudus	5	64	59*	71	78*	91	95*	123	97	138	117	155	146*
		75	79*	85	86*	93	115	116	132	146	140	173	156
H. oreg.	5	58	74	78	87	95	96*	121	101	138	120	—	—
		64	89	73	99	96	105	117	115*	144	130	—	159
H. nudus	15	71	45	76	84*	92	94*	121	91	138	111	—	—
		70	66	78	83*	92	96*	121	114	141	144*	167	159
H. oreg.	15	67	54*	74	73*	88	77	112	91	139	112	—	—
		65	85	78	88	93	100	115	111*	115	130	180	158
H. nudus	25	60	48	76	74*	89	81*	123	97	138	112	—	—
		61		76	79*	90	97	115	112*	140	133	—	156
H. oreg.	25	71	57	76	76*	93	78	109	88	135	112	174	138
		69	82	73	82	90	90*	108	113*	135	127	—	168

* = not significant.

Effect of temperature

Table II compares 48-hour blood and urine concentrations at 5°, 15° and 25° C. for summer and winter animals over a salinity range from 12% to 150% sea water. Blood and urine changes for seasonally adapted crabs have been discussed in the previous section. These data represent, in part, an effect of temperature which is due to seasonal change.

Hemigrapsus oregonensis

At 5° C., below 75%, winter animals showed significantly higher urine concentrations than summer animals. Above this salinity, summer values exceeded winter ones. At 15° C. no difference was noted in low salinities, but at higher salinities, summer values were significantly higher than their winter counterparts. At 25° C. summer values significantly exceeded winter values in all salinities except 25% in which they were the same.

Blood concentrations were hyper-osmotic to all experimental salinities at all temperatures, summer and winter. At salinities less than 100%, blood concentrations of winter animals at 5° and 15° C. were higher than those of summer animals at any of the temperatures. Above this salinity, blood of summer animals at the two lower temperatures was significantly higher when compared with winter crabs. At lower salinities the highest blood concentration, for winter animals in particular, was found generally at the lowest temperature. As the concentration of the experimental salinity decreased, blood concentration increased as temperature decreased.

Hemigrapsus nudus

At 5° C., in salinities of 75% and less, no significant differences were observed between summer and winter urine concentrations. At higher salinities, summer values significantly exceeded winter ones. At 15° and 25° C. summer and winter urine concentrations were similar, in 25% and 75% sea water, but in both lower and higher salinities, summer values were significantly higher.

Blood concentrations were hyper-osmotic to all experimental salinities at all temperatures, summer and winter. Over the salinity range, 6% to 125%, blood concentrations for summer or winter crabs were similar, except for the 5° C. winter data. At the lower salinities, both summer and winter groups, the highest blood concentration generally was found at the lowest temperature, even though the temperature effect on blood concentration at a given salinity was relatively slight for summer animals.

Interspecific comparison

Urine data suggest that *H. oregonensis* shows seasonal differences in concentration, and temperature effect during a given season, over a wider range of conditions than *H. nudus*. The principal differences in response between these two species occurred in salinities between 12% and 75%. Comparison of summer blood of the two species shows this body fluid to be similar over most of the salinity range. Major differences occur only at the higher salinities, where *H. oregonensis* is the higher. *H. nudus* has the higher winter blood concentration over the major

portion of the salinity range. At the lower salinities *H. oregonensis* maintains a higher blood gradient.

Urine-blood ratio (U/B)

Summer U/B ratios approached unity in both species (Table 1). In most of the selected conditions, blood was more concentrated than urine. Where U/B ratios >1.0, the departure from unity was not significant. Blood was significantly hyper-osmotic to urine in *H. oregonensis* in 75% and 125% sea water at 15° C., and in *H. nudus*, only in 12% at 5° C.

Winter U/B ratios in both species in selected conditions were all lower than comparable summer ones. The absolute differences in concentration between urine and blood increased to statistically significant levels in most of these conditions. The increases were due to a generally larger net decrease in urine concentration than blood from summer to winter in similar experimental conditions. Blood values were significantly higher than urine values in all conditions, except for two shown in Table I.

At 5° C., *H. oregonensis*, winter and summer, had higher U/B ratios than *H. nudus* over the entire range of salinities. In winter at 15° and 25° C., and in summer at 25° C for the same salinities, *H. nudus* ratios were higher, but the differences were significant only in winter animals at 25° C.

Discussion

Hyper-osmotic regulation of blood concentration in *H. oregonensis* and *H. nudus* was demonstrated by Jones (1941). Gross (1957a) showed some degree of hypo-osmotic regulation in these species. Recent work (Dehnel, 1962) has demonstrated that the osmoregulatory abilities of the two species changed significantly from summer to winter. The results presented here support and complement the latter findings with details of urine osmotic responses.

Effect of salinity

From an equilibrated or steady-state at time zero (Figs. 1 to 4), the urine osmotic response curves fall in low and rise in high salinities at rates which in general decline with time and reach new equilibria with media within the physiological limits of the species. Blood response curves for *Hemigrapsus* (Dehnel, 1962) and *Pachygrapsus* (Gross, 1957a) exhibit similar patterns. In the examples cited, most of the changes were complete by 24 hours' immersion in the media. In *Emerita*, an adjuster, Gross (1957a) showed that all blood changes were complete after only two hours in a comparable range of experimental salinities.

The antennary glands of *Pachygrapsus* have been shown to function mainly in the regulation of particular blood ions but not of total blood osmotic concentration (Jones, 1941; Robertson, 1949; Prosser, Green and Chow, 1955; Gross, 1957a, 1959). This conclusion was based on the isosmoticity of blood and urine in a variety of temperature and salinity combinations, and on high U/B ratios for magnesium (Gross, 1959). The prawns, *Palaemonetes varians, Leander serratus* and *L. squilla,* in dilute media produce urine isosmotic with blood (Panikkar,

1941). Parry (1954) showed Mg^{++} and SO_4^{--} to be lower in blood than in urine in *L. serratus*. Dehnel (unpublished data) has shown that the antennary glands of *Hemigrapsus* regulate the magnesium ion, which is similar to that reported for *Pachygrapsus*. In both species of *Hemigrapsus*, summer-adapted animals at least have total osmotic U/B ratios close to unity over the entire range of experimental temperature and salinity (Table I). At the same time, large osmotic gradients resulted in salinities below 75% sea water (Fig. 5) and the animals are regulating effectively. No significant weight increase was demonstrated in low salinities (Dehnel, 1962). After an initial rapid drop in urine concentration, the rate of salt loss diminished after 24 hours, and a new equilibrium resulted. Hyper-osmoticity of urine in summer animals exposed to concentrated media can have little adaptive importance, since salinities higher than 35% sea water are not as a rule encountered in this geographic area. Webb (1940) postulates that salt absorption is a continuous process under normal conditions. Hyper-osmotic urine in *Hemigrapsus* may be attributed to the suggested activity of salt-absorbing tissues in the gut and gills, which, when adapted to a high temperature and low salinity, continue to respond as in low salinities.

Three major differences distinguish the blood and urine osmotic responses of summer- and winter-adapted animals of both species, at their respective temperatures, to the range of experimental salinities from 12% to 150% sea water. The first is that over a series of sea water concentrations, winter animals showed a smaller range of urine concentration than summer animals. This was markedly true for *H. oregonensis* (Figs. 1 to 4). *Hemigrapsus nudus*, in winter, showed a reduced tolerance for very low external salinity. Such a reduction, expressed by high mortality, was also shown for *C. crangon*, a migratory shrimp (Broekema, 1941). The second difference was that U/B ratios for winter animals were in most cases significantly lower than summer ratios, because urine was considerably more hypo-osmotic to blood (Table I). This suggests winter participation of the antennary glands in hyper-osmotic regulation. The significance of low U/B ratios is not easy to see in relation to the third and most important difference between winter and summer responses: the production in winter of hypo-osmotic urine in external salinities above 75% sea water for *H. nudus* and above 100% sea water for *H. oregonensis*.

While hypo-osmotic regulation of blood concentration has been well documented for a number of Crustacea from aquatic, intertidal, semi-terrestrial and terrestrial habitats (Broekema, 1941; Jones, 1941; Prosser, Green and Chow, 1955; Gross, 1957a, 1957b; Riegel, 1959), it was not found in *Hemigrapsus* by Jones (1941), whose results have been cited widely. Gross (1957a), however, maintained that some degree of hypo-osmotic regulation of blood concentration occurred in *Hemigrapsus* from California, and gave a value of up to 33% perfect regulation for 20 hours in 150% sea water. Dehnel (1962) has shown that both species of *Hemigrapsus*, equilibrated in 75% sea water, did not maintain blood hypo-osmoticity when transferred to experimental salinities of 100% to 175% sea water. Present results have indicated that although hypo-osmotic regulation of blood was not established, increases in concentration may be resisted to some degree. It was shown that urine may differ in concentration from both blood and media, and that seasonal changes occurred in urine as well as blood osmotic responses.

Summer-adapted *Hemigrapsus* in the field were hyper-osmotic to summer salinities (25% to 35%) and blood and urine were nearly isosmotic. Similar osmotic behavior was found in *Carcinus* in dilute sea water and *Eriocheir* in fresh water (Krogh, 1939). Webb (1940) has suggested that active water uptake is suspended and ion exchanges in gills and antennary glands are intensified under these conditions. The low permeability characteristic of the exoskeleton of regulating forms would aid the animals in resisting the influx of excess water with increasing osmotic gradients (Gross, 1957a).

When exposed to increased or decreased experimental salinities, summer-adapted animals behaved osmotically as if they were still in "normal" summer conditions, although the concentration of their body fluids followed changes in the external medium. In low salinities, both species maintained hyper-osmoticity of blood and urine, and this might be accomplished as Webb (1940) suggested. Another possibility is that during the experimental period, salts are mobilized from adaptive extra-vascular pools, whose existence was postulated by Hukuda (1932) and verified in *Pachygrapsus* by Gross (1958, 1959). These pools in *Hemigrapsus* have not been established. Summer-type regulation, characterized by active ion absorption, and probably by reduced water intake, presumably accompanied by some selective ion reabsorption in the antennary glands, is, however, largely extra-renal and does not change after a period of 48 hours in experimental conditions.

The excretion of urine which is hypo-osmotic to the blood as a means of maintaining blood concentration above that of the medium is well documented. Winter-adapted *Hemigrapsus* in the field showed blood concentrations hyper-osmotic to 70% to 80% sea water, and *H. nudus* had higher blood concentrations than *H. oregonensis* in 75% sea water and 5° C. (Fig. 6). Urine data for winter animals from the field are not available, but after 51 hours (48 equilibration plus 3 experimental) in 75% sea water and 5° C. urine concentrations of the two species were similar and hypo-osmotic to the blood. For comparison with summer data, these values have been considered to approximate the urine and blood relationships in winter animals from field conditions.

Winter animals of both species, in experimental media below average winter sea water concentration, regulated their blood concentration with the production of hypo-osmotic urine. Blood and urine concentrations were significantly higher in *H. oregonensis* than in *H. nudus* in 12% and 25% sea water. Only *H. oregonensis* survived as long as 48 hours in 6% sea water (Figs. 2 and 4). The larger blood-to-medium gradients shown by *H. oregonensis* in 6%, 12% and 25% sea water suggest a more active ion-absorbing mechanism in this species, perhaps correlated with the animal's characteristically estuarine distribution. The active absorption of ions from hypo-osmotic media has been demonstrated in a variety of regulating Crustacea, among them, a crayfish, *Astacus*, and the crabs, *Carcinus* and *Eriocheir*, the latter being related to *Hemigrapsus* (Schwabe, 1933; Nagel, 1934; Krogh, 1939).

In crabs, the gills have been recognized as major sites of absorption (Nagel, 1934; Gross, 1957a; Green, Harsch, Barr and Prosser, 1959). Excess water can enter the animals through the gills by diffusion and by active absorption, together with specific ions. Urine, if formed by filtration, at first may be isosmotic with the blood and be rendered hypo-osmotic by the reabsorption of specific ions.

As long as ion loss in the urine is balanced by active absorption from dilute media, animals can achieve and maintain osmotic equilibrium. Increased urine output in dilute media has been shown to aid in elimination of excess water in *Carcinus* (Prosser and Brown, 1961). It has not been demonstrated in the present data but may be important in *Hemigrapsus* as well.

Effect of temperature

Broekema (1941) reported that *Crangon crangon* maintained in sea water of 29‰ showed a gradual decrease in blood concentration as experimental temperature was allowed to rise with the seasonal change from spring towards autumn (blood-medium gradient gradually increased). A reversal of these changes occurred when the experimental temperature was allowed to fall between autumn and winter. This species, in Dutch waters, winters offshore in water of relatively high salinity and migrates shoreward into more brackish conditions in spring and early summer. Survival at low temperatures was correlated with high salinity, and high temperature increased tolerance to low salinity. Other species, with a reverse migratory pattern, appeared to tolerate low salinities better at low temperatures. These included a spider crab, *Hyas araneus*, a shrimp, *Crangon allmani*, and a prawn, *Pandalus montagui*. A third group, represented by the crab, *Rhithropanopeus harrisi* and the amphipod, *Gammarus duebeni*, had tolerances similar to *Hyas* but did not migrate seasonally (Verwey, 1957). The two species of *Hemigrapsus* combine tolerances similar to *C. crangon*, and non-migratory habits. Dehnel (1960) suggested that low salinities at high temperatures may impose a greater stress than high ones. This is compatible with observed osmotic gradients maintained by these species between blood, urine and media in high and low salinities.

Urine and blood concentrations were alike for summer-adapted *H. oregonensis* in 12% sea water and 15° C. and the gradient between these fluids and the medium was 55% sea water. Cooling the animals at 5° C. reduced this gradient by 10% sea water for urine and 2% for blood. Blood osmotic concentration was regulated as strongly as at 15° C. Urine and blood concentrations were similar at 25° C., but the gradient between them and the medium increased, indicating that summer adaptation favors stronger regulation at high temperatures and low salinities and emphasizes the resemblance of the temperature and salinity tolerances of this species to those of *C. crangon*.

Summer-adapted *H. nudus*, at 5° C. in 25% and 12% sea water, showed urine to be significantly hypo-osmotic to blood (Table I), suggesting here also that the antennary glands are taking part in the elimination of excess water and reabsorption of ions. At 15° and 25° C., in dilute media, urine concentrations were not significantly different from those at 5° C., but blood-to-urine gradients were slightly reduced, suggesting that cooling of summer-adapted animals in low salinity conditions reduced their capacity for salt absorption and stimulated greater reabsorption in the antennary glands to compensate.

Winter-adapted animals of both species in dilute (12%) sea water showed significantly greater urine hyper-osmoticity at 5° than at 15° C. Blood data from winter-adapted animals at 12% and 5° C. are not available for comparison. With

the rise in temperature, the U/B ratio decreased because absolute urine concentration decreased the greater. A rise in experimental temperature from 15° to 25° C. caused no further significant change in urine concentration. In 75% sea water, at 5° C., winter animals of both species showed similar urine concentrations but *H. nudus* had the higher blood concentration, hence a smaller U/B ratio resulted (Table I). At 15° C., *H. nudus* urine remained unchanged, blood concentration dropped, the U/B ratio rose, and regulation weakened. In *H. oregonensis,* however, blood did not change but urine concentration decreased, giving a lower U/B ratio. Thus, in experimental conditions appproximating winter field temperature and salinity, *H. oregonensis* responded to a rise in temperature by a drop in urine concentration, while maintaining blood at the level found at 5° C. This is probably achieved by increased reabsorption in the antennary glands. A further rise in temperature to 25° C. caused no significant change in urine concentration. This species regulates less strongly in 75% sea water as temperature increases from 5° to 15° C. In *H. nudus*, the rise in temperature from 15° to 25° C. resulted in a significant decrease in urine, but not blood, concentration, hence a lower U/B ratio. U/B ratios for the two species in 75% sea water were identical at 25° C. but *H. nudus* had urine and blood values about 7% sea water higher than *H. oregonensis,* indicating somewhat stronger regulation.

The effects on urine concentration of cooling or warming summer-adapted animals and of warming winter-adapted animals were pronounced only in low experimental salinities. In high salinities, similar changes in temperature caused no significant change in urine concentration in either species. It is probable that high salinities pose less of an osmotic problem than low salinities, and that temperature changes consequently do not alter the balance between absorptive and reabsorptive activities as much in high as in low salinities.

SUMMARY

1. Total osmotic pressure measurements of urine were determined on two species of crabs, *Hemigrapsus nudus* and *H. oregonensis,* over a salinity range, 6% to 175% sea water, three temperatures, 5°, 15° and 25° C., and at two seasons, summer and winter. Blood data are included from Dehnel (1962) for comparison.

2. Urine and blood concentrations fall in dilute, and rise in concentrated media, at rates directly related to the gradients between media and equilibrated body fluid concentrations, and are influenced by the seasonal adaptation of the animals and the experimental temperature. Major changes in body fluids occurred within 48 hours.

3. Hyper-osmotic regulation in summer-adapted animals resulted in isosmoticity of blood and urine, implicating extra-renal mechanisms. The production of hypo-osmotic urine in winter-adapted animals indicated the participation of the antennary glands.

4. In both species, summer and winter adaptation tended to favor stronger hyper-osmotic regulation at the respective seasonal temperatures than at temperatures foreign to the seasons.

5. Changes in experimental temperature revealed seasonal and interspecific differences in 48-hour blood and urine concentrations. Blood concentrations of *H.*

oregonensis, when measured at a series of temperatures and salinities, showed a general trend, particularly for winter animals. As the concentration of the experimental media decreased (from 75% to 12%) blood concentrations increased significantly with decreasing temperature. Blood concentrations of summer animals showed no real differences, but when compared with winter crabs, at lower salinities, blood concentrations of summer crabs were significantly lower. The same general trend was shown for *H. nudus*. With respect to urine concentrations, summer-adapted *H. oregonensis*, in dilute media, showed significantly higher urine concentrations at higher temperatures. *H. nudus* showed no temperature effects in any salinities. Winter-adapted animals of both species showed significant decreases in urine concentration in low and intermediate, but not high, salinities, when the experimental temperature was increased.

6. Seasonal adaptation of osmoregulatory mechanisms in *Hemigrapsus* is shown to alter the balance of active processes so that for a given range of experimental conditions, urine is lower in winter animals than in summer, both in absolute concentration and relative to the blood.

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